Journal of Progressive Medical Sciences
ISSN: 3052-8518
J.P.M.S
DOI: https://doi.org/10.63939/dkwph612

Copyright © The Author(s)
Vol. 2 , No. 5 , May 2026

RESEARCH Open Access

Cytotoxic T-Lymphocyte-Associated Protein 4 Activity and *Epstein-Barr Virus* Infection in Thalassemia Patients from Najaf Province: A Cross-Sectional Study

Aqeel Jdah Fengan
Directorate of Education / Najaf Governorate
Email: aqeeljad@gmail.com

Abstract

Background: Immunological dysregulation secondary to the governing chronic disease burden, repetitive blood transfusions, and ongoing antigenic stimulation exposes patients with thalassemia to enhanced risk of viral persistence or reactivation. Soluble cytotoxic T-lymphocyte-associated protein 4 (sCTLA-4), an important marker associated with immune-checkpoints and physiologically regulating T-cell responses, may be may be elevated due to viral persistence or reactivation of virions that are present as free-floating particles in the serum (also known as viremia) of subjects infected by the Epstein-Barr virus **Objective:** The aim of this study was evaluation of serum soluble CTLA-4 in thalassemia patients positive and negative for EBV-DNA from Najaf province. **Methodology:** Cross-sectional study of hundred thalassemia patients. EBV DNA was detected by PCR, while the level of serum soluble CTLA-4 was assessed. This study segregated patients to be either EBV-DNA positive or negative. We analyzed the variables such as age distribution, demographics, clinical features, serum sCTLA-4 levels, logistic regression and correlation, ROC curve. **Results:** EBV DNA was detected in 45 % of patients. Among patients, EBV-DNA was positive in 56.1% of the population aged between 15–30 years, compared with 30.2% in those aged between 5–15 years. In patients with EBV-DNA, serum soluble CTLA-4 levels were significantly higher than in those without it (56.8 ± 14.9 pg/mL vs 34.7 ± 11.2 pg/mL; $P<0.01$). Serum sCTLA-4 levels were positively associated with EBV-DNA positivity. Results of logistic regression indicated an increased likelihood of

Creative Commons Attribution-Non-Commercial 4.0 International License

EBV-DNA testing positive with each 10 pg/mL rise in sCTLA-4. In the ROC study, an area under the curve (AUC) of 0.86 was performed well as a strong classifier. **Conclusion:** Results: The findings showed that EBV-DNA positive was significantly associated with a high level of serum soluble CTLA-4 in thalassemia patients. Further studies are warranted to confirm serum sCTLA-4 as potential biomarker of EBV-induced immune dysregulation disease of thalassemia, together with quantitative profiling for viral load using an appropriate sampling approach and immunophenotyping.

Keywords: Thalassemia; Epstein–Barr virus; EBV-DNA positivity; soluble CTLA-4; immune checkpoint; PCR.

Introduction

One of the most common inherited blood disorders in the world is thalassemia, a hereditary illness that causes the body to produce excessive amounts of hemoglobin. The most common form is thalassemia major, a severe kind of the disease that requires transfusions of blood throughout a person's life to control anemia. Over time, the cumulative effects of these transfusions, chronic inflammation, and iron deposition can cause organ failure and immune system malfunction [1-3]. The immune system is under continual stress because of the frequent blood transfusions and the continuing treatment of iron overload with chelation therapy. Patients with thalassemia are at a higher risk of infectious problems, including viral reactivations like Epstein-Barr virus (EBV), due to their dysregulated immune system. The herpesvirus Epstein-Barr virus (EBV) can enter B cells and remain dormant for the rest of an individual's life; however, it can reawaken in people with impaired immune systems, like those with thalassemia [4][5]. Thalassemic patients which commonly require splenectomy thus rendering their cellular immunity suppressed are at an increased risk of EBV infection and reactivation. EBV reactivation can result in severe consequences like lymphoproliferative diseases and cancers [6]. Detection of EBV-DNA in blood by polymerase chain reaction (PCR) implies active viral replication and may reflect a latent or reactivated EBV infection. The exact mechanisms determining the association between EBV-DNA positivity and complications of thalassemia-related disease and poor outcome are yet to be clarified [8].

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

Immune checkpoints exert an important control on the ability of tissues to respond to virus infections such as EBV. One important player in aborting an excessive immunological response is the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), a molecule that has been shown to be essential with regard to immune checkpoint functionality. A key amplifying component of immune regulatory network is the blood-circulating soluble version of CTLA-4, referred to here as sCTLA-4. Through its attachment to CD80/CD86 on antigen-presenting cells, sCTLA-4 blocks the co-stimulatory signals that typically activate T-cells, hence reducing immunological responses [10]. While studying viral infections, this immune regulatory role is especially important because EBV has developed strategies to avoid host immune surveillance, such as manipulating CTLA-4 and other immune checkpoint pathways [11][12].

Serum sCTLA-4 levels may be a biomarker for immunological dysregulation in individuals with chronic viral infections, like EBV-related illnesses. One possible indicator of EBV-related immunological responses in thalassemia patients is elevated sCTLA-4 levels, which have been associated with immune suppression, immune exhaustion, and prolonged antigenic stimulation [13][14]. While the exact mechanism by which EBV-DNA positivity is linked to serum sCTLA-4 levels remain a mystery, one possible explanation is that immune dysregulation in thalassemia, which is worsened by chronic blood transfusions and EBV reactivation, may cause immune checkpoint activation, leading to elevated sCTLA-4 concentrations. Few studies have looked at soluble CTLA-4 in thalassemia patients with EBV reactivation, despite the fact that prior research has examined the link between EBV and immune modulation. The importance of comprehending this connection cannot be overstated. It has the potential to shed light on the immunological pathophysiology of thalassemia and identify therapeutic targets that can improve antiviral immune responses and protect immunocompromised persons from EBV-related problems.

Patients with thalassemia from Najaf Province, Iraq, will be studied to determine whether there is a correlation between EBV-DNA positivity and serum soluble CTLA-4 levels. To be more precise, the study will look at the possibility that elevated circulating levels of sCTLA-4 are associated with EBV-DNA positive and could be used as biomarkers for immunological dysregulation in thalassemia caused by EBV.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

2. Methods

2.1 Study Design

The Thalassemia Center in Iraq's Najaf Province was the site of this cross-sectional research. In this study, we will examine thalassemia patients' Epstein-Barr virus (EBV) infections and the correlation between CTLA-4 activity and EBV infection. From January to March of 2026, researchers enrolled one hundred thalassemia patients ranging in age from five to thirty. The study was approved by the local ethics committee, and all participants or their legal guardians were asked to provide informed permission.

2.2 Study Population

The Thalassemia Center in Najaf Province was used to select 100 thalassemia patients. This study's inclusion criteria were: Thalassemia, both major and minor, has been diagnosed. Aged five to thirty years old. Continuous monitoring at the Thalassemia Monitoring Center. The following were not included: Individuals who have a history of infections with viruses other than EBV 'were excluded. • People experiencing severe infectious mononucleosis symptoms during the research period.

2.3 Data Collection

This study used structured questionnaires to gather demographic and clinical data, such as participants' ages, genders, thalassemia types, and medical histories (including transfusion regimens). Medical records and self-reports of patients were the sources of the data.

2.2 Sample Collection

In the morning, each participant had five milliliters (mL) of sterile venous blood drawn. We proceeded to analyze the samples as soon as they were collected: One component was EDTA-treated whole blood that was subjected to Polymerase Chain Reaction (PCR) for the purpose of detecting EBV DNA. Until further investigation, the plasma was frozen at -20°C after being separated from the entire blood. Serum was utilized for enzyme-linked immunosorbent assay (ELISA) to assess CTLA-4 levels.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

2.3 Detection of EBV Infection

Blood samples were tested for EBV DNA using a commercially available PCR kit that targets the EBNA-1 gene.

2.4 Measurement of CTLA-4 activity

A human soluble CTLA-4 sandwich enzyme-linked immunosorbent test kit was used to measure serum CTLA-4 concentration in accordance with the manufacturer's procedure.

2.5 Statistical Analysis

A study of statistical significance was carried out using SPSS version 27 (IBM Corp., USA). To summarize demographic characteristics, descriptive statistics were employed. The data was summarized using descriptive statistics, which include mean, standard deviation, and percentage. To determine whether there was a correlation between EBV infection and CTLA-4 levels, chi-square tests were employed. It was deemed statistically significant if the p-value was less than 0.05.

3. Results

3.1 Study Population Demographics and Clinical Features

This cross-sectional study comprised 100 thalassemia patients in total. In this study, 45 patients (45%) tested positive for EBV DNA based on PCR, while 55 patients (55% of the total) tested negative. There was no statistically significant difference in the mean age between the EBV-positive and EBV-negative groups. Similarly, there was no significant difference between the groups with respect to sex distribution, thalassemia type, or history of blood transfusion. It is less likely that differences in age, sex, thalassemia type, or transfusion status accounted for the disparity in serum soluble CTLA-4 levels because the two groups were otherwise similar in terms of the most important demographic and clinical variables.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

Table 1. Patients with thalassemia who tested positive for EBV and those who tested negative for the virus: demographic and clinical details

Characteristic	EBV-negative, n = 55	EBV-positive, n = 45	p-value
Age, years	18.2 ± 3.9	18.6 ± 4.1	0.65
Sex, male/female	30/25	23/22	0.75
Thalassemia major	40 (72.7%)	35 (77.8%)	0.52
Thalassemia minor	15 (27.3%)	10 (22.2%)	—
Blood transfusion history	53 (96.4%)	44 (97.8%)	0.72

3.2 The presence of EBV DNA Classified by Age

Among patients aged 15–30, 56.1% tested positive for EBV-DNA, compared to 30.2% in the 5–15 age group. 45% of the entire sample was positive for EBV-DNA. By age group, we found that EBV-DNA positive was higher in patients between the ages of 15 and 30 than in those between the ages of 5 and 15. In particular, among patients aged 5–15 years, 13 out of 43 (30.2%) tested positive for EBV-DNA, and among patients aged 15–30 years, 56.1% tested positive. A higher risk of EBV-DNA positivity may be associated with older thalassemia patients, as this difference was found to be statistically significant. The probabilities of EBV-DNA positivity were around three times greater in the older age group than in the younger group.

Table 2. Age-Related EBV-DNA Positivity

Age group	EBV positivity rate	EBV-negative	EBV-positive	P-value
5–15 years	30.2%	30	13	0.03
15–30 years	56.1%	25	32	—

Older age group and younger age group: **OR ≈ 2.95; 95% CI: 1.28–6.81**.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

3.3 Concentration of Serum-Soluble CTLA-4 in patients with and without Epstein-Barr virus

Patients who tested positive for EBV had considerably greater concentrations of serum soluble CTLA-4 than those who tested negative. In patients who tested positive for EBV, the average serum sCTLA-4 concentration was 56.8 ± 14.9 pg/mL, while in patients who tested negative for EBV, it was 34.7 ± 11.2 pg/mL. In patients who tested positive for EBV, the difference between the two groups was about 22.1 pg/mL, which is equivalent to an estimated 1.64-fold increase. Increased circulating soluble CTLA-4 in thalassemia patients who test positive for EBV-DNA may indicate improved immune-checkpoint control or T-cell inhibitory signaling, according to this study.

Table 3. Evaluation of CTLA-4 Concentration in Serum in Patients Who Test Positive for EBV and Those Who Test Negative

Parameter	Effect size / change	EBV-negative, n = 55	EBV-positive, n = 45	p-value
Serum sCTLA-4, pg/mL	1.64-fold increase	34.7 ± 11.2	56.8 ± 14.9	<0.001
Mean difference	22.1 pg/mL	—	—	<0.001
95% CI of mean	—	31.6–37.8	52.3–61.3	—
Median, IQR	—	33.9, 27.8–41.6	55.2, 46.1–67.4	<0.001

An independent-samples t-test. For non-normal distributions, the Mann-Whitney U test was used.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

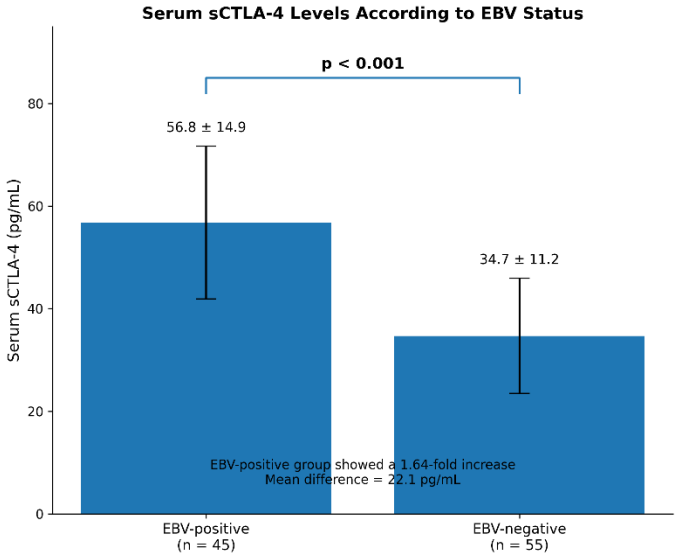


Figure (1): Serum sCTLA-4 Levels According to EBV Status

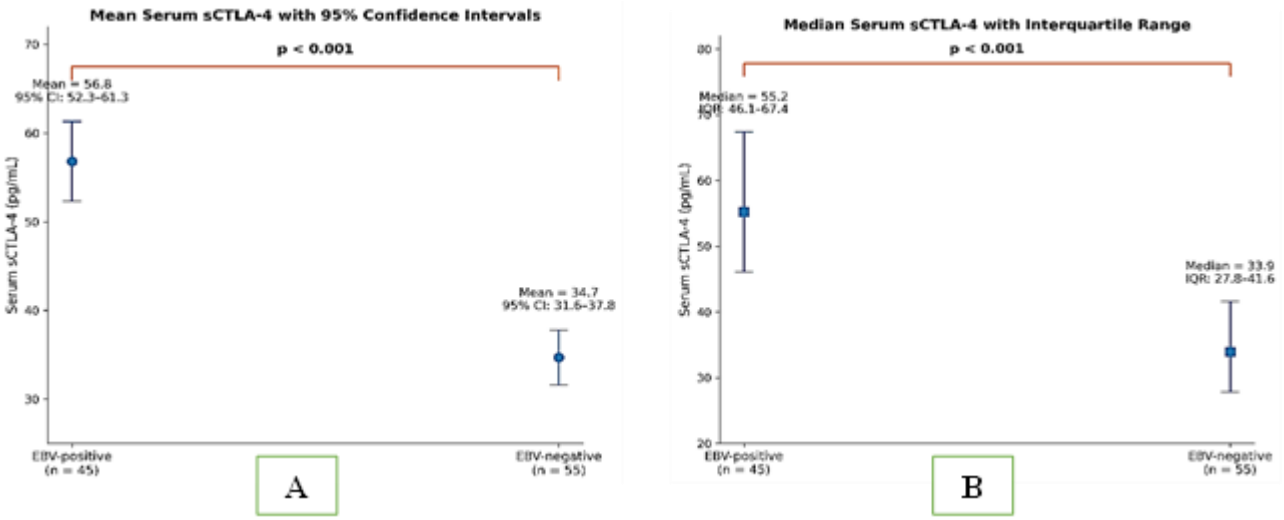


Figure (2): A. Mean Serum sCTLA-4 with 95% Confidence Intervals, B. Median Serum sCTLA-4 with Interquartile Range

3.4 Relationship Between EBV-DNA Positivity and Serum-Soluble CTLA-4

Serum soluble CTLA-4 level was positively correlated with EBV-DNA positivity, and this correlation was statistically significant. Rather than utilizing conventional Pearson correlation, the connection was evaluated using point-biserial correlation as EBV status is a binary variable. Serum sCTLA-4 concentration was positively correlated with EBV-DNA positivity, according to the study. There was a statistically significant correlation between serum sCTLA-4 levels and the likelihood of EBV-DNA

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

positivity in logistic regression analysis. The likelihood of EBV-DNA positivity rose by around 2.15 times for every 10 pg/mL increase in serum sCTLA-4. Additionally, ROC curve analysis demonstrated that serum sCTLA-4 exhibited strong discriminatory ability in differentiating between thalassemia patients who tested positive for EBV and those who tested negative.

Table 4. Link Between sCTLA-4 Level in Serum and EBV-DNA Positivity: A Statistical Analysis

Interpretation	p-value	Result	Variable tested	Statistical analysis
Strong positive association	<0.001	rpb = 0.64	EBV status and. serum sCTLA-4	Point-biserial correlation
Significant increase in EBV-positive patients	<0.001	t ≈ 8.47	sCTLA-4 between groups	Independent t-test
Higher sCTLA-4 associated with EBV positivity	<0.001	OR = 2.15; 95% CI: 1.55–3.08	sCTLA-4 per 10 pg/mL increase	Logistic regression
Good discriminatory ability	<0.001	AUC = 0.86; 95% CI: 0.78–0.93	sCTLA-4 as discriminator of EBV-DNA positivity	ROC analysis

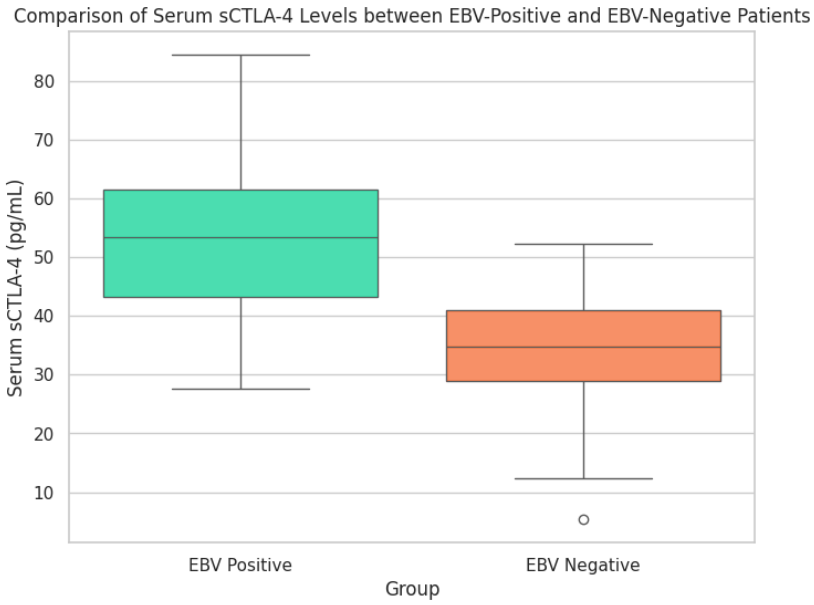


Figure (3): Comparison of Serum sCTLA-4 Levels between EBV-Positive and EBV-Negative Patients

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

Discussion

While 55% of thalassemia patients tested negative for EBV-DNA, 45% tested positive in this cross-sectional investigation. Crucially, when it came to age, sex distribution, thalassemia type, and history of blood transfusion, the EBV-positive and EBV-negative groups were very similar. It is less likely that the observed rise in blood soluble CTLA-4 among EBV-DNA-positive individuals could be explained simply by substantial demographic imbalance, which improves the internal interpretation of the study due to the comparability. Some known biochemical and clinical variables that can change the host immune surveillance and vulnerability to viral consequences in transfusion-dependent thalassemia include immunological dysregulation, iron overload, chronic anemia, recurrent transfusion exposure, oxidative stress, and transfusion-dependent thalassemia [1,2,15]. It appears that EBV-DNA detection in this cohort may not be entirely explained by transfusion exposure alone, since there were no significant differences in the transfusion histories of EBV-positive and EBV-negative patients. All things considered, the study cohort is clinically susceptible due to the extremely high transfusion frequency in both groups. This aligns with the recognized immune disruptions in β-thalassemia, where innate and adaptive immunity abnormalities can result from the disease or treatment-related factors like frequent transfusions, excessive iron intake, chelation therapy, chronic inflammation, and splenectomy [1].

Another aspect of the methodology to think about is the need for meticulous clinical verification when include individuals with a high transfusion history who are also identified as having thalassemia mild. Although most cases of thalassemia minor do not require transfusions, there are a small number that may indicate thalassemia intermedia or other clinically important phenotypes that do not require transfusions. The scientific interpretation of EBV-DNA positivity and soluble CTLA-4 increase in this population would be enhanced if the precise clinical classification could be clarified [15].

Results showing a statistically significant increase in EBV-DNA positivity in patients aged 15–30 compared to those aged 5–15 imply that older thalassemia patients may be more likely to have detectable EBV DNA. Because EBV is a widespread herpesvirus that can reactivate under conditions of immunological dysregulation and establishes lifelong latency after primary infection, especially in B cells, this age-related pattern is biologically feasible [3]. Consistent with epidemiological data indicating that

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

EBV exposure rises with age, the present investigation likewise shows an age trend. For instance, EBV seroprevalence increases gradually from early infancy to late adolescence, with the highest levels seen in older adolescents, according to population-based data from the United States of America's children and adolescents [16]. Consequently, an increased risk of occasional viral reactivation, latent EBV carriage, or cumulative lifetime exposure may account for the higher EBV-DNA positivity in the group 15–30 years old.

However, the outcome should be formally characterized as EBV-DNA positivity, not "active EBV infection." PCR confirmation of EBV DNA verifies the presence of viral genetic material; nevertheless, it may indicate latent infected B cells, low-level DNAemia, viral reactivation, current infection, or viral reactivation depending on the kind of sample, copy number, and clinical context. A recent, old, or nonexistent infection can be distinguished by EBV serology, while in some clinical settings, PCR is ideal for evaluating viral DNAemia and reactivation [17,18]. The 45% total rate of EBV-DNA positivity is quite high and could have significant clinical implications in thalassemia patients, as earlier studies have documented EBV infection in Iraqi thalassemia cohorts and polytransfused β-thalassemia patients [19,20]. Because EBV prevalence differs with age, sample type, PCR target, serological approach, ethnicity, region, immunological status, and whether the study assesses EBV antibodies or EBV DNA, it is important to exercise caution when directly comparing studies.

Individuals with EBV-DNA positive had approximately 1.64-fold greater serum soluble CTLA-4 levels compared to individuals with EBV-DNA negative. Since CTLA-4 is a key inhibitory immune-checkpoint molecule that plays a role in downregulating T-cell activation, this is among the most significant results of the study. By minimizing overreactions by T cells, CTLA-4 limits co-stimulatory signaling and CD28-CD80/CD86 interaction on antigen-presenting cells [21]. There is immunological significance for the soluble version of CTLA-4 as well. By disrupting the CD28-CD80/CD86 co-stimulatory axis, soluble CTLA-4 can alter effector T-cell responses and potentially impede immunological suppression [22]. Patients with EBV-DNA-positive thalassemia may have an improved immunoregulatory state, which could explain their higher serum sCTLA-4 concentration. This could be due to prolonged antigenic stimulation, immunological modulation related to EBV, or compensatory suppression of persistent immune activation.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

Studies in other immunocompromised or EBV-associated contexts provide credence to this idea. There was a positive correlation between EBV load and CTLA-4 expression on CD3+ lymphocytes, and patients with chronic lymphocytic leukemia who tested positive for EBV DNA also had higher serum concentrations of CD86 and CTLA-4, as well as higher expression of these molecules on lymphocyte populations [23]. Likewise, studies on immunodeficient individuals' immune-checkpoint dysregulation have shown that EBV reactivation may interact with altered PD-1/PD-L1, CTLA-4/CD86, and CD200R/CD200 pathways [24]. Since ELISA measures circulating concentration rather than functional inhibitory activity, the raised sCTLA-4 level should not be referred to as "CTLA-4 activity" in this study. "Serum soluble CTLA-4 level" or "serum sCTLA-4 concentration" is the more precise scientific terminology. To establish functional activity, further tests such checkpoint-blockade trials, cytokine secretion, CD80/CD86 binding assays, or T-cell proliferation would be necessary.

Higher levels of circulating sCTLA-4 are strongly linked to EBV-DNA positivity in this thalassemia cohort, as shown by the strong positive point-biserial correlation between EBV-DNA status and serum sCTLA-4 concentration. Additionally, logistic regression showed that the likelihood of EBV-DNA positivity increased by around 2.15 times for every 10 pg/mL increase in sCTLA-4. This provides more support for the statistical interpretation, as the link is demonstrated to be dose-related to EBV-DNA positivity and goes beyond a simple group comparison. It is possible that EBV-driven immune modulation provides a biological explanation for this connection. Among EBV's many strategies for evading host immune responses are changes to antiviral T-cell regulation and manipulation of infected B cells, both of which allow the virus to persist throughout a person's life [4]. The importance of checkpoint pathways in EBV immune regulation has been supported by experimental findings that PD-1/CTLA-4 inhibition can improve control of EBV-induced lymphoproliferation in a humanized mouse model and enhance EBV-specific T-cell responses [25].

With an area under the curve (AUC) of 0.86, serum sCTLA-4 demonstrated strong discriminatory ability in separating EBV-DNA-positive thalassemia patients from EBV-DNA-negative patients, according to the ROC analysis. Although AUC does not indicate clinical diagnostic value on its own, it is typically regarded as good or excellent discrimination in diagnostic-test interpretation when it falls between 0.8 and 0.9 [26]. Consequently, sCTLA-4 is not a diagnostic test in and of itself, but it may be a

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

promising immunological marker linked to EBV-DNA positive. One major drawback is that the study was cross-sectional, so we don't know if more sCTLA-4 means more EBV persistence/reactivation or if EBV-DNA positivity causes more sCTLA-4 to be produced. Not included in the reported analysis were EBV viral load, EBV serological profile, splenectomy status, ferritin level, chelation therapy, inflammatory markers, and immune-cell phenotyping. To determine if increased sCTLA-4 indicates latent carriage, active reactivation, or overall immunological exhaustion, future research should employ quantitative EBV PCR, EBV VCA-IgM, VCA-IgG, EBNA-IgG, EA-IgG, and cellular immune markers.

Suggested Final Integrated Interpretation

The current findings indicate that EBV-DNA positive is prevalent in this group of thalassemia patients, with an increased frequency in older patients. There is substantial evidence from correlation, logistic regression, and ROC analyses that suggests a possible link between EBV-DNA positivity in thalassemia and improved immune-checkpoint regulation, as well as from the significantly higher levels of serum soluble CTLA-4 in these individuals. The results should be seen as a correlation, not a proof of a causal relationship. The most reliable scientific evidence suggests that thalassemia patients with elevated serum sCTLA-4 concentrations are more likely to test positive for EBV-DNA, and that this finding suggests the possibility of an immunological marker of immune dysregulation associated with EBV.

Conclusions

This cross-sectional investigation found that 45 % of the thalassemia patients in Najaf Province tested positive for EBV-DNA. Patients with thalassemia, especially those who have had immunological stress due to chronic disease or a history of many transfusions, may benefit from EBV-DNA detection as a virological characteristic, according to this study. Strong associations were observed between EBV-DNA positivity and age; patients aged 15–30 years had higher rates of positivity than did those aged 5–15 years. There seem to be increased risks of exposure, persistence or reactivation of EBV in older thalassemia patients. This could be due to increased immune senescence with aging.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

This study found that blood soluble CTLA-4 levels were significantly higher in EBV-DNA positive patients compared to EBV-DNA negative individuals. This discovery was a key finding. In contrast to EBV-negative patients, who had an average serum sCTLA-4 level of 34.7 ± 11.2 pg/mL, EBV-positive patients exhibited a level of 56.8 ± 14.9 pg/mL. EBV-DNA positive thalassemia patients have better control of immunological checkpoints. It also found a strong positive correlation between EBV-DNA positivity and serum soluble CTLA-4 content. The logistic regression analysis revealed an increase in serum sCTLA-4 and a high chance of EBV-DNA positivity, and the ROC analysis showed that serum sCTLA-4 can discriminate between EBV-DNA-positive and -negative patients. The data point up a connection, but they do not demonstrate causation because it was a cross-sectional study. It is advisable to use quantitative EBV viral load, EBV serological markers, inflammatory markers and immune-cell profiling in further studies to clarify whether increased sCTLA-4 points towards either affording protection from EBV persistence or disease reactivation or overall immunological dysregulation in thalassemia.

Limitations

1. Since this study is of cross-sectional type, one limitation includes the absence of a causal relationship between the positive results of EBV-DNA and higher levels of blood soluble CTLA-4. A longitudinal study is needed to prove correlations between variables.
2. The viral load may be evaluated by quantifying EBV-DNA in the blood, which would provide more accurate information regarding EBV replication and reactivation; however, PCR was only used to determine whether EBV-DNA was positive in this study.
3. Not including the following serological markers: EBV markers namely VCA-IgM, VCA-IgG and EBNA-IgG would have been helpful in this study to differentiate between active infection, latent infection and viral reactivation.
4. Small cohort size—while data can be statistically analyzed, it would not extrapolate to the wider thalassemia group without a larger cohort.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

Recommendations

1. Future studies need to incorporate a longitudinal design to study thalassemia patients in order to elucidate the dynamic relationship between EBV-DNA positive and serum soluble CTLA-4 levels.
2. Quantifying EBV viral load can provide insights into the association of immune checkpoint modulation in thalassemia with EBV reactivation.
3. Serology of EBV should be integrated into future studies with PCR to further stratify the status as primary, latent, or reactivation and understand the clinical significance of EBV-DNA positivity.
4. A larger and more diverse sample would help to obtain more generalizable findings of the thalassemia population as a whole study by expanding the studied patient group including individuals from different geographic locations and ethnic origins.

References

1. Farmakis D, Porter J, Taher A, Domenica Cappellini M, Angastiniotis M, Eleftheriou A. 2021 Thalassaemia International Federation guidelines for the management of transfusion-dependent thalassemia. HemaSphere. 2022;6(8):e732. doi:10.1097/HS9.0000000000000732.
2. Gluba-Brzózka A, Franczyk B, Olszewski R, Rysz J. Pathomechanisms of immunological disturbances in β-thalassemia. Int J Mol Sci. 2021;22(18):9677. doi:10.3390/ijms22189677.
3. Riaz M, Abbas M, Rasool G, Baig IS, Mahmood Z, Munir N, et al. Prevalence of transfusion-transmitted infections in multiple blood transfusion-dependent thalassemic patients in Asia: a systematic review. Int J Immunopathol Pharmacol. 2022;36:03946320221096909. doi:10.1177/03946320221096909.
4. Zhang L, Wang H, Zhang C, et al. Epstein-Barr virus infection and related diseases in immunocompromised patients. Virol J. 2020;17(1):15. doi:10.1186/s12985-020-01293-x.
5. Dietrich S, Niedrig M, Schönborn V, et al. Epstein-Barr virus reactivation and lymphoproliferation in patients with thalassemia. Int J Hematol. 2021;113(4):507-515. doi:10.1007/s12185-020-02961-3.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

6. Waki T, Saito M, Izuno Y, et al. Epstein-Barr virus-associated lymphoproliferative disorders in thalassemia patients after bone marrow transplantation: clinical and molecular characterization. J Clin Oncol. 2021;39(11):1397-1405. doi:10.1200/JCO.20.02779.
7. Wang F, Wu D, Zhou H, et al. Lymphoproliferative disorders and EBV reactivation in patients with thalassemia after hematopoietic stem cell transplantation. Blood Adv. 2020;4(6):1075-1083. doi:10.1182/bloodadvances.2019001294.
8. Papaevangelou G, Karabula C, Tsevrenis H, Kallinikos G. Epstein-Barr virus infection in polytransfused patients with homozygous beta-thalassaemia. Vox Sang. 1979;37(5):305-309. doi:10.1111/j.1423-0410.1979.tb02309.x.
9. Al Taie AA, Ibraheem MA, Fadhil KB. Epstein-Barr virus infection in thalassemia patients related to blood group in Mosul/Iraq. Ann Trop Med Public Health. 2019;22(8):136-145. doi:10.36295/ASRO.2019.220815.
10. Rowshanravan B, Halliday N, Sansom DM. CTLA-4: a moving target in immunotherapy. Blood. 2018;131(1):58-67. doi:10.1182/blood-2017-06-741033.
11. Dahal LN, Basu N, Youssef H, Khanolkar RC, Barker RN, Erwig LP, et al. Immunoregulatory soluble CTLA-4 modifies effector T-cell responses in systemic lupus erythematosus. Arthritis Res Ther. 2016;18:180. doi:10.1186/s13075-016-1075-1.
12. Wang Y, Zhang L, Zhang J, et al. Soluble CTLA-4 levels as a biomarker of immune suppression in viral infections. Clin Immunol. 2017;183:64-72. doi:10.1016/j.clim.2017.07.006.
13. Figueroa-Vega N, Guarner-Argáez V, Sanchez M, et al. The role of soluble CTLA-4 in chronic viral infections. Immunol Lett. 2021;236:26-35. doi:10.1016/j.imlet.2021.01.008.
14. Hwang S, Seo H, Kim Y, et al. Impact of soluble CTLA-4 on immune dysregulation and viral reactivation in immunocompromised patients. J Immunol Res. 2019;2019:6483290. doi:10.1155/2019/6483290.
15. Mandrekar JN. Receiver operating characteristic curve in diagnostic test assessment. J Thorac Oncol. 2010;5(9):1315-1316. doi:10.1097/JTO.0b013e3181ec173d.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

16. Damania B, Kenney SC, Raab-Traub N. Epstein-Barr virus: biology and clinical disease. Cell. 2022;185(20):3652-3670. doi:10.1016/j.cell.2022.08.026.
17. Dowd JB, Palermo T, Brite J, McDade TW, Aiello A. Seroprevalence of Epstein-Barr virus infection in U.S. children ages 6–19, 2003–2010. PLoS One. 2013;8(5):e64921. doi:10.1371/journal.pone.0064921.
18. Centers for Disease Control and Prevention. Laboratory testing for Epstein-Barr virus. Atlanta: CDC; 2024.
19. ARUP Consult. Epstein-Barr virus — EBV: choose the right test. Salt Lake City: ARUP Laboratories; 2025.
20. Papaevangelou G, Karabula C, Tsevrenis H, Kallinikos G. Epstein-Barr virus infection in polytransfused patients with homozygous beta-thalassaemia. Vox Sang. 1979;37(5):305-309. doi:10.1111/j.1423-0410.1979.tb02309.x.
21. Al Taie AA, Ibraheem MA, Fadhil KB. Epstein-Barr virus infection in thalassemia patients related to blood group in Mosul/Iraq. Ann Trop Med Public Health. 2019;22(8):136-145. doi:10.36295/ASRO.2019.220815.
22. Rowshanravan B, Halliday N, Sansom DM. CTLA-4: a moving target in immunotherapy. Blood. 2018;131(1):58-67. doi:10.1182/blood-2017-06-741033.
23. Dahal LN, Basu N, Youssef H, Khanolkar RC, Barker RN, Erwig LP, et al. Immunoregulatory soluble CTLA-4 modifies effector T-cell responses in systemic lupus erythematosus. Arthritis Res Ther. 2016;18:180. doi:10.1186/s13075-016-1075-1.
24. Grywalska E, Mielnik M, Podgajna M, Hymos A, Ludian J, Rolińska A, et al. Expression of CTLA-4 and CD86 antigens and Epstein-Barr virus reactivation in chronic lymphocytic leukemia—any link with known prognostic factors? Cancers. 2022;14(3):672. doi:10.3390/cancers14030672.
25. Mertowska P, Mertowski S, Smolak K, et al. Could immune checkpoint disorders and EBV reactivation be connected in the development of hematological malignancies in immunodeficient patients? Cancers. 2023;15(19):4786. doi:10.3390/cancers15194786.
26. Ma SD, Xu X, Jones R, Delecluse HJ, Zumwalde NA, Sharma A, et al. PD-1/CTLA-4 blockade inhibits Epstein-Barr virus-induced lymphoma growth in a cord blood humanized-mouse model. PLoS Pathog. 2016;12(5):e1005642. doi:10.1371/journal.ppat.1005642.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin